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(54) METHOD AND APPARATUS FOR CONDUCTING AN ARRAY OF CHEMICAL REACTIONS ON A SUPPORT SURFACE

VERFAHREN UND VORRICHTUNG ZUR DURCHFÜRUNG EINER VIELZAHL VON CHEMISCHEN REAKTIONEN AUF EINER TRAEGERFLAECHE

PROCEDE ET APPAREIL POUR REALISER UNE SERIE DE REACTIONS CHIMIQUES SUR UNE SURFACE DE SUPPORT

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- **SCIENCE, vol.251, 1991 pages 767 - 773 FODOR, S.P.A. ET AL. 'LIGHT-DIRECTED, SPATIALLY ADDRESSABLE PARALLEL CHEMICAL SYNTHESIS' cited in the application**

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Description

BACKGROUND OF THE INVENTION

This is a continuation-in-part of Serial No. 07/754,614 filed September 4, 1991, pending.

Field of the Invention

The invention relates to methods for conducting a large number of chemical reactions on a support surface, methods for making the support surface, and the support surface itself.

Summary of the Related Art

Proposals for the direct sequencing of DNA by hybridization with arrays of oligonucleotides are known in the art. Drmanac et al., Genomics 4; 114 (1989) proposes hybridization array-mediated DNA sequencing by binding target DNA to a dot blot membrane, followed by probing with an array of oligonucleotides. Khrapko et al., FEBS Letters 256, 118 (1989) proposes hybridization array-mediated DNA sequencing by binding the oligonucleotide array to a support membrane, followed by probing with target DNA.

Synthesis of arrays of bound oligonucleotides or peptides is also known in the art. Houghton, in the Multiple Peptide System product brochure describes the T-bag method, in which an array of beads is physically sorted after each interaction. This method becomes unwieldy for the preparation of large arrays of oligonucleotides. Geysen et al., J. Immunol. Methods 102; 259 (1987) discloses the pin method for the preparation of peptide arrays. The density of arrays that may be produced by this method is limited, and the dipping procedure employed in the method is cumbersome in practice. Southern, Genome Mapping and Sequencing Conference, May 1991, Cold Spring Harbor, N.Y., disclosed a scheme for oligonucleotide array synthesis in which selected areas on a glass plate are physically masked and the desired chemical reaction is carried out on the unmasked portion of the plate. In this method it is necessary to remove old mask and apply a new one after each interaction. Fodor et al., Science 251; 767 (1991) describes a method for synthesizing very dense 50 micron arrays of peptides (and potentially oligonucleotides) using mask-directed photochemical deprotection of synthetic intermediates. This method is limited by the slow rate of photochemical deprotection and by the susceptibility to side reactions (e.g., thymidine dimer formation) in oligonucleotide synthesis. Khrapko et al, FEBS Letters 256; 118 (1989) suggests simplified synthesis and immobilization of multiple oligonucleotides by direct synthesis on a two dimensional support, using a printer-like device capable of sampling each of the four nucleotides into given dots on the matrix. However, no particulars about how to make or use such a device are provided.

Some methods for permanently attaching oligonucleotides to glass plates in a manner suitable for oligonucleotide synthesis are known in the art. Souther, Chem. abst. 113; 152979r (1990) describes a stable phosphate ester linkage for permanent attachment of oligonucleotides to a glass surface. Mandenius et al., Anal. Biochem. 157; 283 (1986) teaches that the hydroxyalkyl group resembles the 5'-hydroxyl of oligonucleotides and provides a stable anchor on which to initiate solid phase synthesis.

The related art contains numerous ideas and information related to arrays of chemical reactants on a solid support. However, existing or suggested methods are limited, and do not conveniently and reliably produce the very large, high density arrays. There is, therefore, a need for new methods for preparing large high density arrays of reactive sites. Ideally, such methods should utilized relatively simple machinery to produce large, dense arrays of solid phase bound reactants in a reproducible and rapid manner.

SUMMARY OF THE INVENTION

This invention provides a method for conducting a large number of chemical reactions on a support surface. Solutions of chemical reactants are added to functionalized binding sites on the support surface by means of a piezoelectric pump. This pump deposits microdroplets of chemical reactant solution onto the binding sites. The chemical reactant at each binding site is separated from the others by surface tension. Typically, the support surface has $10\text{-}10^4$ functionalized binding sites per cm^2 and each functionalized binding site is about 50-2000 microns in diameter. Typically, the amounts of reagents added to each binding site is in a volume of about 50 picoliter to 2 microliter. The reactions at the functionalized binding site may form covalent bonds such as esters or amide bonds or may involve non-covalent specific binding reactions such as antibody/antigen binding or oligonucleotide specific binding. The invention also includes array plates and methods for making the array plates.

Typically, the array plates are made by the process set out in Figure 2A by

(a) coating a support surface with a positive or negative photoresist substance which is subsequently exposed and developed to create a patterned region of a first exposed support surface;